Waters
Lab Highlights

An Internal Communication
of Applications and Techniques

LAH 0364A 6/88
AN/SP/--/--/--

Gradient Solvent Delivery Performance
I. Introduction and Definitions

(Replacement for LAH 0364 2/88)

This Lab Highlight is the first in a series which will consider the topic of gradient solvent delivery performance. The series will describe methods for running test procedures to establish baseline levels of system performance, and will describe configurations for obtaining optimum performance in specialized applications.

In order to obtain optimum performance in gradient liquid chromatography, solvent delivery systems must provide both accurate and precise flow rates and proportioning of solvents as well as adequate mixing of the mobile phase components. Since Waters offers several types of configurations to form gradients, it may be confusing as to what is "the best way." In fact, there is no one "best way." There are many ways to configure a system, involving some tradeoffs, any of which may be "the best" for a particular application. To understand these tradeoffs, first it is important to define the terms used to describe solvent delivery systems and gradient performance.

The term "accuracy" refers to the closeness of the measured value of flow or mobile phase composition to the indicated (or theoretical) value which is set on the instrument. For example, if a specification of ± 1% is published for flow accuracy, and the pump is set at 1.0 mL/min, the actual measured value will fall between 0.99 and 1.01 mL/min. In statistical terms, accuracy refers to the closeness of the mean of measured values to the true value of the thing measured. It is very important that the flow rate be the same each time the instrument is operated, and this will be the case if there is high precision.

Table 1

	M6000A	M590/510	W600
Flow Accuracy	± 1%[1]	± 1%	n.r.
Flow Precision	n.r.[2]	± 0.1%	± 0.1%
Compositional Accuracy	n.r.[2]	n.r.[3]	± 0.5%
Compositional Precision	n.r.[3]	n.r.[3]	± 0.15%

n.r. -- Not reported in manual.

[1] Incorrectly termed "Flow Precision" in manual.

[2] R.P.W. Scott in *J. Chrom.* **138**, 283-307 demonstrated flow precision values of ± 0.07% for the M6000A. (The 590/510 are mechanically the same.)

[3] Not reported, must be determined using multiple pumps. The M6000A is no longer manufactured, but is essentially the same as the M510.

"Precision" refers to the reproducibility of a measurement. If a measurement is precise, each individual measurement will differ from the mean value by only a small amount. In statistics this means that the standard deviation is small. In practical terms, high precision (indicated by a small percentage error) means that flow rates and mobile phase proportioning will be consistent each time the solvent delivery system is used. A precision of ± 0.1% for a pump accurately delivering 1.0 mL/min means that each time a flow rate of 1.0 mL/min is set, the flow will range from 0.999 to 1.001 mL/min. This precision is extremely important if reproducible chromatography is to be obtained. The accuracy and precision of Waters solvent delivery systems is evident from published specifications (Table 1), and has been attested to in the technical literature.[1]

In gradient chromatography, the solvent delivery system must combine two or more solvents to produce a mobile phase having the proper composition. This process will be termed "proportioning" in these articles. Precise proportioning is required to assure reproducible retention times and peak heights.

Finally, provision is made for the mixing of the mobile phase components. When one of the mobile phase components has a significant absorbance at the wavelength being monitored by a UV detector, minute irregularities in the mixing of the components will be greatly amplified. In this special situation a "rippling" baseline is observed. This rippling may be unacceptable to some scientists if the ripples are as large as some of the analyte peaks. It is possible to remove this small amount of mixing irregularity by adjusting the "mixing volume."

Mixing is accomplished in the so-called "mixing" volume between the point of combination of the mobile phase components and the head of the column. Thorough mixing may be achieved by use of large mixing volumes, but there will be a considerable time delay between the actual start of the gradient and the point at which the beginning of the gradient reaches the head of the column. In addition, large mixing volumes result in exponential-type dilution which is manifested as a "rounding off" of rapid compositional changes such as step gradients.

If an attempt is made to reproduce manufacturer's specifications, there is a recommended mixing configuration which must be used for each Waters gradient solvent delivery system (see the appropriate manual), which provides reasonable delay volumes and mixing for most gradient applications. Some applications (e.g. gradient separations of peptides involving trifluoroacetic acid) may require additional mixing for smooth baselines and other applications (e.g. small dimension or microbore columns) may require shorter delays. Each of these "unique" situations may require the LC system to be "uniquely" configured, even though this configuration may depart from the "standard" configuration described in the manual.

Future articles in this series* will describe 1) a simple procedure which may be run to measure certain characteristics of a gradient solvent delivery system, 2) an illustration of the effects of varying the mixing configuration, and 3) certain application-specific configurations.

*Also see "Rapid Quantitative Analysis by Gradient Elution Chromatography," J. Korpi & B. Bidlingmeyer, American Lab., June 1981, p. 110, for a thorough discussion.

[1] R.P.W. Scott and C. E. Reese, *J. Chrom.* 138, 238-307 (1977). (Quoted in Lab Highlight 0034, January 1982.)

* * *